ANIMAL CELL, METHOD FOR PRODUCING ANIMAL CELL, AND METHOD FOR PRODUCING TARGET PROTEIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a Continuation of PCT International Application No. PCT/JP2019/048216 filed on Dec. 10, 2019, which claims priority under 35 U.S.C § 119(a) to Japanese Patent Application No. 2018-231592 filed on Dec. 11, 2018. Each of the above application(s) is hereby expressly incorporated by reference, in its entirety, into the present application.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention relates to animal cells that express a target protein. An aspect of the present invention relates to a method for producing the above-mentioned animal cells, and a method for producing a target protein using the above-mentioned animal cells.

2. Description of the Related Art

[0003] In producing biopharmaceuticals such as antibodies, fed-batch culture, which improves a state of cells by adding nutrients to a culture solution, is often used to improve the productivity of antibodies. In addition, in producing biopharmaceuticals such as antibodies, a perfusion culture method, in which a culture solution is continuously filtered and discharged while a fresh medium containing nutrients is continuously fed to a culture tank, is often used to improve the productivity of antibodies.

[0004] WO2009/020144 discloses a method for producing polypeptide, including strongly expressing alanine aminotransferase, culturing cells in which DNA encoding a desired polypeptide is introduced, and producing the desired polypeptide, as a method capable of producing protein in a high production amount.

SUMMARY OF THE INVENTION

[0005] In fed-batch culture, it is possible to perform cell culture for a long period and at a higher cell density compared to a case where nutrients are not additionally added, but there is a problem in that cell viability is degraded in the latter half of the culture due to accumulation of waste products secreted from cells, and thus products cannot be increased by extended culture period.

[0006] In addition, there is a problem in that in order to culture cells at high density in the perfusion culture method, the cost of the culture is increased since harvesting and supplying of a medium in an amount of 1 to 3 times the culture volume per day is required to supply nutrients and discharge waste products out of the system.

[0007] An object of an aspect of the present invention is to provide an animal cell capable of producing a target protein with high productivity. Another object of an aspect of the present invention is to provide a method for producing the animal cell and a method for producing a target protein using the animal cell.

[0008] The present inventors have conducted intensive studies to achieve the above-mentioned objects, and as a result, the present inventors found that the production amount of protein is affected by forcibly expressing a sodium-dependent neutral amino acid transporter-2 (SNAT2) gene (gene name is SLC38A2) in CHO cells, and thus it is possible to achieve improvement of antibody productivity (Qp) and improvement of antibody production amount. Aspects of the present invention was completed based on the above findings.

[0009] That is, according to an aspect of the present invention, the following inventions are provided.

[0010] <1> An animal cell that has a gene encoding a target protein and a foreign gene encoding SNAT2 and linked to a promoter, and overexpresses the SNAT2.

[0011] <2> The animal cell according to <1>, in which the overexpression is constant.

[0012] <3> The animal cell according to <1> or <2>, in which the foreign gene encoding SNAT2 has a base sequence having 90% or more sequence identity with a base sequence of SEQ ID NO: 1.

[0013] <4> The animal cell according to any one of <1> to <3>, in which the foreign gene encoding SNAT2 includes a base sequence of SEQ ID NO: 1.

[0014] <5> The animal cell according to any one of <1> to <4>, in which the animal cell is a CHO cell.

[0015] <6> A method for producing the animal cell according to any one of <1> to <5>, the method including a step of introducing a gene encoding a target protein and a foreign gene encoding SNAT2 and linked to a promoter, into an animal cell.

[0016] <7> The method according to <6>, in which the step of introducing a foreign gene encoding SNAT2 and linked to a promoter is performed by electroporation.

[0017] <8> A method for producing a target protein, including culturing the animal cell according to any one of <1> to <5>.

[0018] <9> The method according to <8>, in which the culture is fed-batch culture.

[0019] <10> The method according to <9>, in which a seeded cell density of a cell culture is 0.2×10^6 cells/mL to 5×10^6 cells/mL.

[0020] <11> The method according to <10>, in which a viable cell rate during a culture period is 60% or more over the entire period.

[0021] <12> The method according to <8>, in which the culture is perfusion culture.<

[0022] 13> The method according to <12>, in which the seeded cell density of the cell culture is 0.2×10^6 cells/mL to 1×10^7 cells/mL.

[0023] <14> The method according to <13>, in which a viable cell rate during a culture period is 90% or more over the entire period.

[0024] According to the animal cell of an aspect of the present invention, it is possible to produce a target protein with high productivity.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. **1** illustrates a result of measuring an antibody concentration in a culture supernatant of cells after gene introduction.

[0026] FIG. **2** illustrates a result of measuring antibody productivity (Qp) per cell in the culture supernatant of cells after gene introduction.

[0027] FIG. **3** illustrates a result of measuring a cell size after gene introduction.